



EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of a health claim related to 3 g/day plant stanols as plant stanol esters and lowering blood LDL-cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006

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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to 3 g/day plant stanols as plant stanol esters and lowering blood LDL-cholesterol and reduced risk of (coronary) heart disease pursuant to Article 14 of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Raisio Nutrition Ltd, submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of Finland, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to 3 g/day plant stanols as plant stanol esters per day and lowering blood LDL-cholesterol by 12 % and reduced risk of (coronary) heart disease. The applicant has further requested that the minimum duration to obtain the effect be stated to be one to two weeks, and that the claims be authorised for an extended range of foods, including yellow fat spreads, dairy products, cheese, rye bread, oatmeal, fermented soy milk based products (drinkable and spoonable yoghurt-type products), and oat based milk drinks. The applicant provided an unpublished meta-analysis with 18 randomised, controlled human studies on the LDL-lowering efficacy of plant stanol esters at intakes between 2.7 to 3.3 g per day plant stanols. On the basis of the data presented, the Panel concludes that plant stanol esters at a daily intake of 3 g plant stanols (range 2.7 g to 3.3 g) in matrices approved by Regulation (EC) No 376/2010 (yellow fat spreads, dairy products, mayonnaise and salad dressings) lowers LDL-cholesterol by 11.4 % (95% CI: 9.8 – 13.0), that the minimum duration required to achieve the maximum effect of plant stanol esters on LDL-cholesterol lowering is two to three weeks, and that while plant stanol esters added to foods such as margarine-type spreads, mayonnaise, salad dressings, and dairy products such as milk, yoghurts including low-fat yoghurts, and cheese have been shown consistently to lower blood LDL-cholesterol levels, the size of the cholesterol-lowering effect of plant stanols added to other food formats is less well established.

¹ On request from the Competent Authority of Finland following an application by Raisio Nutrition Ltd, Question No EFSA-Q-2011-00851, adopted on 26 April 2012.

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KEY WORDS

Plant stanols, plant sterols, coronary heart disease, LDL-cholesterol

SUMMARY

Following an application from Raisio Nutrition Ltd, submitted pursuant to Article 14 of Regulation (EC) No 1924/2006 via the Competent Authority of Finland, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to 3 g plant stanol esters per day and lowering blood LDL-cholesterol by 12 % and reduced risk of (coronary) heart disease. The applicant has further requested that the minimum duration for the effect be stated to be one to two weeks, and that the claims be authorised for an extended range of foods, including yellow fat spreads, dairy products, cheese, rye bread, oatmeal, fermented soy milk based products (drinkable and spoonable yoghurt-type products), and oat based milk drinks.

The Panel notes that the applicant's request specifically relates to the amendment of an already authorised claim laid down in Regulation (EC) No 376/2010 so that it would refer to an intake of 3.0 g/d of plant stanol esters added to an extended range of foods which would lead to an LDL-cholesterol lowering effect of 12 % within 1 - 2 weeks. The application included a request for the protection of proprietary data.

The applicant provided an unpublished meta-analysis on 18 randomised, controlled human studies on the LDL-lowering efficacy of plant stanols (as plant stanol ester) at intakes between 2.7 to 3.3 g per day in matrices approved by the European Commission. In this analysis the relative pooled LDL-cholesterol lowering effect was 11.4 % (95% CI, 9.8 - 13.0 %). The Panel notes some limitations of the applicant's meta-analysis that contribute to uncertainty of the estimate of the LDL-cholesterol lowering effect (e.g. the study quality not being taken into account, and the estimation of the relative net changes of LDL-cholesterol levels and variance parameters which were not reported in most of the original articles).

The applicant claimed that the minimum duration required for efficacy was one to two weeks. In two of three treatment arms, in which the earliest LDL-cholesterol measurement was made after one week of intervention with plant stanol ester enriched foods, the maximum LDL-cholesterol lowering effect was achieved already after one week of intervention; in the third study the maximum effect was achieved after two intervention weeks. In four out of eight treatment arms in which the earliest LDL-cholesterol measurement was made after two weeks of the plant stanol ester intervention the maximum LDL-cholesterol lowering effect was attained after two weeks of intervention, while in the four other treatment arms most of the reduction was already achieved after two weeks. The Panel considers that the minimum duration required to achieve the maximum effect of plant stanols on LDL-cholesterol lowering is two to three weeks.

With regards to the proposed conditions of use, the applicant suggested that the food matrices should not be limited to those specified in Regulation (EC) No 376/2010 (yellow fat spreads, dairy products, mayonnaise and salad dressings) and that the claimed effect can also be achieved with other matrices (rye bread, oatmeal, fermented soy milk based products (drinkable and spoonable yoghurt-type products), and oat based milk drinks). The Panel notes that all of the 18 treatment arms analysed in the meta-analysis used matrices specified in Commission Regulation (EC) No 376/2010 and that no human intervention study has been provided by the applicant to demonstrate that 3 g plant stanols consumed with other food matrices can lower blood LDL-cholesterol by 12 %.

On the basis of the data presented, the Panel concludes that plant stanol esters at a daily intake of 3 g plant stanols (range 2.7 g to 3.3 g) in matrices approved by Regulation (EC) No 376/2010 (yellow fat spreads, dairy products, mayonnaise and salad dressings) lowers LDL-cholesterol by 11.4 % (95% CI: 9.8 – 13.0), that the minimum duration required to achieve the maximum effect of plant stanol esters on LDL-cholesterol lowering is two to three weeks, and that while plant stanol esters added to foods such as margarine-type spreads, mayonnaise, salad dressings, and dairy products such as milk, yoghurts including low-fat yoghurts, and cheese have been shown consistently to lower blood LDL-

cholesterol levels, the size of the cholesterol-lowering effect of plant stanols added to other food formats is less well established.

The Panel could have reached these conclusions without considering the unpublished meta-analysis claimed as proprietary by the applicant.

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BACKGROUND

Regulation (EC) No 1924/2006⁴ harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Articles 14 to 17 of this Regulation lay down provisions for the authorisation and subsequent inclusion of reduction of disease risk claims and claims referring to children's development and health in a Community list of permitted claims.

According to Article 15 of this Regulation, an application for authorisation shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA

- The application was received on 29/06/2011.
- The scope of the application was proposed to fall under a health claim referring to disease risk reduction.
- The scientific evaluation procedure started on 10/07/2011.
- On 12-14/10/2011 and on 13-15/12/2011, the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application, and the clock was stopped on 10/11/2011 and 19/12/2011 in compliance with Art. 16(1) of Regulation (EC) No 1924/2006.
- On 05/12/2011 and on 30/12/2011, EFSA received the requested information as submitted by the applicant and the clock was restarted.
- During its meeting on 25-27/04/2012, the NDA Panel, after having evaluated the overall data submitted, adopted an opinion on the scientific substantiation of a health claim related to 3 g/day plant stanols as plant stanol esters and lowering blood LDL-cholesterol by 12 % and reduced risk of (coronary) heart disease.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16 of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to 3 g plant stanol esters and lowering blood LDL-cholesterol by 12 % and reduced risk of (coronary) heart disease.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of plant stanol esters, a positive assessment of its safety, nor a decision on whether plant stanol esters is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

⁴ European Parliament and Council (2006). Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union OJ L 404, 30.12.2006. Corrigendum OJ L 12, 18.1.2007, p. 3–18.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 17 of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: Raisio Nutrition Ltd. P.O. Box 101 (Rasionkaari 55), FI-21201 Raisio, Finland.

The application indicates confidential and proprietary data. The applicant claims confidentiality and proprietary rights for the data related to composition, product certificate of analysis properties and physical properties, batch to batch analyses, manufacturing process, stability information, for an unpublished study report (Hallikainen et al., 2010) of a published study (Hallikainen et al., 2011) and an unpublished meta-analysis which has been conducted on published studies.

Food/constituent as stated by the applicant

Plant stanol ester produced by esterification of plant stanols with fatty acids derived from food-grade vegetable oils or blends of vegetable oils. According to the applicant, the maximum proportion of campestanol from total plant stanols is 32 % in the products related to this application.

Health relationship as claimed by the applicant

Consumption of 3 g/day plant stanols (as plant stanol ester) reduces LDL-cholesterol by 12 %. Reducing LDL-cholesterol reduces the risk of coronary heart disease.

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: "The daily consumption of 3 g plant stanols in ester form has been shown to reduce blood cholesterol by 12 %. High cholesterol is a risk factor in the development of coronary heart disease."

Specific conditions of use as proposed by the applicant

The target population is broad and includes male and female adults with elevated (not optimal) LDL-cholesterol levels who need and want to lower their blood cholesterol, currently receiving or not receiving pharmacological therapy for dyslipidaemia.

To achieve a LDL-cholesterol reduction of 12 %, 3 g of plant stanols (as plant stanol ester) must be consumed daily. The foods to which plant stanol ester are added are commonly consumed as part of the daily diet. Currently marketed products with plant stanol ester include yellow fat spreads, dairy products, cheese, rye bread, oatmeal, fermented soy milk based products (drinkable and spoonable yoghurt-type products), and oat based milk drinks. Given the variety of plant stanol ester containing foods available, the consumption of 3 g/day plant stanols, either as a single dose or in divided doses, can be reasonably consumed as part of a balanced diet.

According to the applicant, the minimum duration required for efficacy is 1 to 2 weeks. The LDL-cholesterol lowering efficacy of plant stanol ester in matrices other than those specified in Regulation (EC) No 376/2010 is comparable to the efficacy of matrices approved for quantitative plant stanol ester claims; thus, the food matrices for plant stanol ester should not be limited to yellow fat spreads, dairy products, mayonnaise, and salad dressings.

As per Regulation (EC) No 608/2004, foods and food ingredients with added phytosterols, phytosterol esters, phytostanols and/or phytostanol esters shall have an "easily visible and legible statement that the product may not be nutritionally appropriate for pregnant and breastfeeding women and children under the age of 5 years".

ASSESSMENT

1. INTRODUCTION

The applicant has proposed the health claim “The daily consumption of 3 g plant stanols in ester form has been shown to reduce blood cholesterol by 12 %. High cholesterol is a risk factor in the development of coronary heart disease.” The applicant has further requested that the minimum duration for the effect be stated to be 1-2 weeks, and that the claims be authorised for a range of foods, including yellow fat spreads, dairy products, cheese, rye bread, oatmeal, fermented soy milk based products (drinkable and spoonable yoghurt-type products), and oat based milk drinks. The Panel notes that a similar health claim has already been authorised by Regulation (EC) No 376/2010 amending Regulation (EC) No 983/2009. The authorised claim is: ‘Plant stanol esters have been shown to lower/reduce blood cholesterol. High cholesterol is a risk factor in the development of coronary heart disease.’ The conditions of use of the claim are: ‘Information to the consumer that the beneficial effect is obtained with a daily intake of 1.5 – 2.4 g plant stanols. Reference to the magnitude of the effect may only be made for foods within the following categories: yellow fat spreads, dairy products, mayonnaise and salad dressings. When referring to the magnitude of the effect, the entire range ‘7 to 10 %’ and the duration to obtain the effect ‘in 2 to 3 weeks’ must be communicated to the consumer’.

The Panel notes that the applicant’s request specifically relates to the amendment of the authorised claim, i.e. that the intake of 3.0 g/d of plant stanol esters added to an extended range of foods leads to an LDL-cholesterol lowering effect of 12 % within 1 - 2 weeks.

The Panel recalls the conclusions from its earlier opinion on plant sterols/stanols and lowering LDL-cholesterol (EFSA, 2009) that (i) the efficacy for lowering LDL-cholesterol is similar for plant sterols and plant stanols, that (ii) 1.5 - 1.9 g and 2.0 - 2.4 g plant sterols/plant stanols per day was observed to lower blood LDL-cholesterol by an average of 8.5 % and 8.9 %, respectively, and that for an intake of 1.5 - 2.4 g/d an average reduction of between 7 and 10.5 % can be expected, that (iii) the blood LDL cholesterol lowering effect is usually established within the first 2 - 3 weeks and can be sustained by a continued consumption of plant sterols/stanols, and that (iv) while plant sterols/stanols added to foods such as margarine-type spreads, mayonnaise, salad dressings, and dairy products such as milk, yoghurts and cheese have been shown consistently to lower blood LDL-cholesterol, the efficacy of plant sterols/stanols added to other food formats is less well established.

This opinion focuses on the efficacy of 3.0 g per day of plant stanol esters on LDL-cholesterol lowering, on the minimum duration for the effect to occur, and on the efficacy in different food matrices.

Throughout this opinion, quantities of sterols and stanol esters are expressed as the equivalent weights of free [i.e. un-esterified] stanols.

2. BLOOD LDL-CHOLESTEROL LOWERING EFFECT OF A DAILY INTAKE OF 3 G PLANT STANOLS AS PLANT STANOL ESTERS

The applicant conducted two literature searches in May 2009 and February 2010 accessing 16 sources (Medline®, CAB Abstracts, Allied & Complementary Medicine, MANTIS™, Pascal, Foodline®: Science, Global Health, Biosis Previews®, Elsevier Biobase, National Technical Information Service, Gale Group Health & Wellness DatabaseSM, EMBASE, New England Journal of Medicine, Adis Clinical Trials Insight, Web of Science, Cochrane Library). Keywords related to plant sterols and stanols (phytosterols), sitosterol, sitostanol, campesterol, campestanol, stigmasterol, brassicasterol, blood lipids, cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and human studies.

The screening of titles, abstracts and full text articles of in total 3,456 references resulted in the identification of 17 treatment arms from 15 studies reported by 12 publications and two unpublished study reports which were considered pertinent by the applicant.

Inclusion criteria were: randomised controlled trial (parallel or crossover) studies conducted in human adults (age >19 years); subjects in the treatment arm received “usual phytosterols” defined as 4-desmethylsterols and/or 4-desmethylstanols produced from vegetable or plant oils such as soybean oil, rapeseed oil, and tall oil; the active treatment consisted of plant stanol esters (i.e. esterified plant stanols comprising ≥ 85 % of total free and esterified plant stanols); for plant stanol/plant sterol blends, the proportion of plant stanols was 85 % or more of the plant stanol/plant sterol blend; the plant stanol ester was manufactured by (i.e. either provided by or purchased from) Raisio Nutrition Ltd.; the target intake administered was between 2.7 to 3.3 g/day of plant stanols (as plant stanol ester); the duration of treatment was a minimum of 2 weeks; fasting plasma/serum LDL-cholesterol was measured either as a primary or secondary outcome variable; relevant blood lipid data were reported or could be calculated; that is, for parallel trials, change from baseline in LDL-cholesterol was reported or could be calculated for both the placebo and treatment groups, and for crossover studies, LDL-cholesterol at the end of the placebo and active treatments was reported or could be calculated; if a co-intervention was administered in the active treatment arm, the co-intervention was such that the effects of the plant stanol ester on LDL-cholesterol could be isolated (i.e. the placebo group was administered the other intervention, e.g. a statin only). Exclusion criteria were: study was not a randomised controlled trial; study was not conducted in adults (where adults is defined as age >19 years; study results were reported in abstract form only; studied subjects included colectomised patients or subjects who were either homo- or heterozygous for sitosterolemia; the control group was not administered a placebo; the active treatment consisted of ferulated phytosterols, such as rice bran oil and/or shea nut oil phytosterols; the active treatment consisted of free plant stanols (i.e. esterified plant stanols comprised < 85 % of total free and esterified plant stanols); for plant stanol/plant sterol blends, the proportion of plant stanols was less than 85 % of the plant stanol/plant sterol blend; the plant stanol ester was not manufactured by Raisio Nutrition Ltd; the target dose administered was not quantified or was < 2.7 or > 3.3 g/day of plant stanols (as plant stanol ester); the duration of the treatment was < 2 weeks; relevant endpoints were not related to fasting LDL-cholesterol; relevant blood lipid data were not reported: that is, for parallel trials, change from baseline in LDL-cholesterol was not reported and could not be calculated for both the placebo and treatment groups, and for crossover studies, LDL-cholesterol at the end of the placebo and active treatments was not reported and could not be calculated; the treatment combined plant stanol ester with other nutritional or pharmaceutical interventions, the effects of which could not be isolated; the study was a kin (duplicate) publication.

Seventeen randomised controlled treatment arms derived from 12 publications met the selection criteria and were analysed via meta-analyses (Raisio, 2011). Eight of the controlled treatment arms were derived from cross-over designs; nine of the treatment arms were from parallel studies. In total 974 subjects were studied. The average age across the 17 treatment arms was 54.2 ± 6.7 years and the average fasting baseline LDL-cholesterol was 3.6 ± 0.7 mmol/L (≈ 139 mg/dL). The study populations included healthy men and women with a mean age ranging from 36 to 58 years (Miettinen et al., 1995 treatment arms 1 and 2; Weststrate and Meijer, 1998 treatment arm 2; Nguyen et al., 1999 treatment arms 1 and 2; Mensink et al., 2002; Cater et al., 2005: treatment arm 2 of study 1 and study 2; Alhassan et al., 2006; Mensink et al., 2010); men and women on stable statin therapy (Blair et al., 2000); men with non-insulin dependent diabetes mellitus (Gylling and Miettinen, 1994; Gylling and Miettinen, 1996 treatment arms 1 and 2); post-menopausal women with previous myocardial infarction (Gylling et al., 1997 study 1); men with a positive history for CHD and on stable statin therapy (Cater et al., 2005 study 3); and men and women with Type 1 diabetes and receiving statins for hypercholesterolemia (Hallikainen et al., 2011).

The Panel noted that in the original application the applicant had excluded from the meta-analysis the study by Homma et al. (2003) which showed a 6.6 % LDL-cholesterol lowering effect with 3g plant

stanols on the basis of the exclusion criterion “plant stanol esters not manufactured by Raisio Nutrition Ltd”. In response to a request for scientific justification for the exclusion of this study the applicant argued that the plant stanol ester preparation used in the Homma-study differed in the source of the plant sterols, in its (60:40) ratio of sitostanol:campestanol (different from Raisio’s plant stanol esters which would not contain more than 32 % campestanol) and also in the production process (i.e. esterification process) which would lead to higher amounts of isomerisation and polymerisation of unsaturated fatty acids (e.g. 6.2 % trans PUFA in the Homma-preparation versus 1 % in the Raisio plant stanols). The Panel noted that the source of the sterols and the ratio between the sitostanol and campestanol do not appear to have a relevant impact on the size of the blood LDL-cholesterol lowering effect (EFSA, 2009) and considers that this also applies to the small differences in the content of *trans* fatty acids (7.2 % versus 1 %). The Panel also noted that the study by Homma et al. (2003) was included by the applicant for its analysis of the continuous dose-response and of the “minimum intervention required to achieve the claimed effect”. The Panel therefore requested the applicant to include the 3 g/day plant stanol arm of the study by Homma et al. (2003) in the meta-analysis of 3 g plant stanol ester studies.

In all of the 18 treatment arms (including Homma et al., 2003) plant stanol esters were used at a daily intake of 3 g or slightly less. Seventeen of the 18 treatment arms used spreadable fats as a vehicle and one used yoghurt. The duration of the intervention ranged from 24.5 to 360 days, with the majority (n = 12) of treatment arms assessing efficacy within 4 to 7 weeks of the intervention. Compliance was reported for nine treatment arms and ranged between 74 and 100 %.

Since baseline LDL-cholesterol is known to significantly affect the degree of absolute LDL-cholesterol lowering (Demonty et al., 2009; Musa-Veloso et al., 2011), the applicant suggested that the more appropriate value is the relative reduction in LDL-cholesterol, as baseline LDL-cholesterol is accounted for in the relative LDL-cholesterol reduction. When relative (%) placebo-adjusted reduction changes and associated variance data were reported in the published articles, these values were used. For studies in which relative net changes were not reported, they were calculated from the absolute changes.

The Comprehensive Meta-Analysis Software Version 2.2.050 was used to conduct the meta-analysis. The inverse of the variance of the placebo-adjusted mean relative change in LDL was used as a weighting factor. In order to obtain variance measures for each study a number of choices were made and different methods were applied, based also on what information was available in the original papers. Although there might be variability in the level of accuracy of standard deviation estimates (subsequently used to calculate variances), due to different sources (calculated in the original study, subsequently derived, estimated via Taylor series approximation), linearised approximations for variance are a well-established methodology in case of missing information on summary statistics. However, the Panel notes the uncertainty deriving from the fact that measures of variability had to be computed/estimated for most of the studies (only for 4 treatment arms were the reported original relative reductions and associated measures of variability used) and that no sensitivity analysis was performed to test the robustness of the imputation of variability parameters (SDs for variance calculation). Random effects models were used, given that there was significant heterogeneity between the studies as indicated by Hedge’s q ($q = 27.49$, $df = 16$, $p = 0.036$ and $q = 29.23$, $df = 16$, $p = 0.022$, respectively).

All treatment arms were controlled for a number of potential confounding variables including age, baseline LDL-cholesterol levels, health/disease status and medication use; sixteen of the treatment arms were controlled for diet, seven treatment arms for alcohol use, four treatment arms for smoking, and one treatment arm for physical activity.

Amongst the 18 treatment arms included in the meta-analyses described above, placebo-adjusted LDL-cholesterol reductions ranged from 5.8 % (Nguyen et al., 1999 treatment arm 1) to 20.4 %

(Alhassan et al., 2006). The pooled relative reduction in LDL-cholesterol was 11.4 % (95% CI, 9.8 - 13.0 %).

The applicant conducted a second *post hoc* meta-analysis without the treatment arm (Nguyen et al., 1999) which showed the lowest (5.8 %) relative LDL-cholesterol reduction among all treatment arms. The applicant reasoned that in this study conducted in the United States, the treatment arm in which a European margarine plant stanol formulation was evaluated had limitations such as a lower compliance (74 % versus 88.9 % for the US margarine plant stanol formulation arm and 78.2 % for the placebo arm). The applicant also argued that the different formulations of the margarines provided different daily amounts of saturated fatty acids: 4.11 g (European margarine) versus 2.25 g (US margarine) versus 2.76 g (placebo margarine) and different daily amounts of poly-unsaturated fatty acids in the margarine: 3.51 g (European margarine) versus 5.22 g (US margarine) and 5.34 g (placebo margarine). After exclusion of the EU margarine-treatment arm of the Nguyen-study, the pooled relative reduction in LDL-cholesterol of the 17 treatment arms was 11.9 % (95% CI, 10.60 - 13.19 %). The applicant noted that the exclusion of this treatment arm of the Nguyen et al. (1999) study eliminated heterogeneity in the meta-analysis, as indicated by hedge's *q*, which was 13.92 (*P* = 0.532), and so the fixed effects model was retained. The Panel considers that the small differences in the fatty acid composition of the margarines would not affect the study outcome. The Panel notes that sensitivity analysis is appropriate when there are outliers but that conclusions should be based on all-studies analyses, and that the results should be reported. *Post-hoc* exclusion of a study based on its relative contribution to the pooled effect size bears the risk of biasing the overall results. The Panel considers that the treatment arm with the European margarine plant stanol formulation should be included in the meta-analysis of plant stanol ester studies with 3 g plant stanols per day. The Panel also notes that the effect of inclusion or exclusion of the aforementioned treatment arm of the study by Nguyen et al. (1999) indicates the sensitivity of the meta-analysis to the inclusion or exclusion of individual studies.

The Panel notes some limitations of the applicant's meta-analysis that contribute to uncertainty of the estimate of the LDL-cholesterol lowering effect. The study quality of the individual studies was not appraised, which was justified by the applicant by referring to the Demonty et al. (2009) meta-analysis (141 treatment arms) where it was concluded that the overall study quality, randomization methods and subject compliance had no impact on the parameter estimates of the dose-response curve. The Panel considers that this conclusion cannot be extrapolated to a small subset of studies included in the meta-analyses by the applicant. Uncertainties derive also from the estimation of the relative (%) net changes of LDL-cholesterol levels and variance parameters which were not reported in most of the original publications.

The Panel also notes that the results of the unpublished meta-analysis provided by the applicant are comparable to the results of the published meta-analysis by Katan et al. (2003) which showed a pooled mean reduction of LDL-cholesterol levels of 11.3 % (95 % CI: 10.2 - 12.3) for ≥ 2.5 g plant stanols and sterols consumed daily. The meta-analysis by Katan et al. (2003) comprised 21 treatment arms (13 stanol arms, 8 sterol arms; average dose 3.0 g) from five studies with doses between 2.5 and 2.9 g, 13 studies with doses between 3.0 and 3.4 g, and two studies with 4.0 and 4.2 g phytosterols, respectively.

In order to provide additional evidence that a 12 % reduction of LDL-cholesterol can be achieved by 3 g of plant stanols per day, the applicant provided another meta-analysis of randomised controlled trials which analysed the LDL-cholesterol lowering efficacy of plant stanols and plant sterols over a continuous dose range (Musa-Veloso et al., 2011). In total, 57 plant stanol ester treatment arms met predefined inclusion and exclusion criteria. The relationship between intake of plant stanol esters and reduction in LDL-cholesterol was modelled using a first-order elimination curve and fit using the nonlinear regression procedure in SAS version 9.0 (SAS Institute), as described by Demonty et al. (2009). The dose-response assessment was conducted on LDL-cholesterol measures obtained at the time point closest to 4 weeks. Using an equation of the first-order elimination function, the authors

retrieved predicted absolute and relative reductions in LDL-cholesterol over a continuous dose range. The Panel notes that the dose-response relationship is fitted using average values from the various studies. This approach does not take the full variability/uncertainty into consideration. As a consequence, the curve does not take into account the full range of variability from individual dose-response, and the 'true' variability/uncertainty of the overall dose-response relationship is underestimated. Additionally, the correlation between observed and predicted reductions is only moderate, suggesting a limited fitting of the model. In fact, the number of observed reductions upon which the curve is modelled for higher doses is very small. There is also uncertainty as to what extent the imbalance in the amount of evidence (i.e. numbers of studies) across the entire dose range affects the shape of the plant stanols curve. The Panel considers that this dose-response analysis does not provide useful information for estimating the cholesterol lowering effect of 3 g of plant stanols in addition to that obtained from the individual studies and the applicant's unpublished meta-analysis.

The Panel considers that the evidence from randomised controlled human intervention studies of plant stanol esters at intakes ranging from 2.7 g to 3.3 g/d in matrices approved by Regulation (EC) No 376/2010 (yellow fat spreads, dairy products, mayonnaise and salad dressings) shows an average pooled LDL-cholesterol reduction of 11.4 % (95% CI: 9.8 – 13.0).

The Panel could have reached this conclusion without considering the unpublished meta-analysis claimed as proprietary by the applicant.

3. MINIMUM DURATION FOR EFFECT OF PLANT STANOL ESTERS

The applicant claims that the minimum duration required for efficacy was one to two weeks. To determine the minimum duration for efficacy, the applicant searched the 57 plant stanol ester treatment arms identified by Musa-Veloso et al. (2011) and identified 11 treatment arms in which LDL-cholesterol measurements were made within 2 weeks following the administration of plant stanol ester enriched foods. In two (Mensink et al., 2002; Noakes et al., 2005) of three treatment arms, in which the earliest LDL-cholesterol measurements was made after one week, the maximum LDL-cholesterol lowering effect was achieved already after one week of intervention; in the third study (Jones et al., 2000) the maximum effect was achieved after two intervention weeks with plant stanols.

In four (Nguyen et al., 1999, treatment arm 1; Blair et al., 2000; Homma et al., 2003, treatment arms 1 and 2) out of eight treatment arms in which the earliest LDL-cholesterol measurements were made after two weeks of the plant stanol ester intervention the maximum LDL-cholesterol lowering effect was attained after two weeks of intervention. In the four other treatment arms (Hallikainen and Uusitupa, 1999, treatment arms 1 and 2; Nguyen et al., 1999, treatment arms 2 and 3) most of the reduction was already achieved after two weeks.

The Panel notes that in most of the studies blood LDL-cholesterol was measured the first time only after two or three weeks of intervention when the full effect has usually been established. The Panel considers that there is insufficient evidence to conclude that the maximum effect is generally achieved already after one week and refers to its previous opinion from 2009 when it concluded that the blood LDL-cholesterol lowering effect is usually established within the first two to three weeks of consumption of stanols (EFSA, 2009).

The Panel considers that the minimum duration required to achieve the maximum effect of plant sterols and stanol esters on LDL-cholesterol lowering is two to three weeks.

4. EFFICACY OF PLANT STANOL ESTERS IN DIFFERENT FOOD MATRICES

With regards to the proposed conditions of use, the applicant suggested that the food matrices should not be limited to those specified in Regulation (EC) No 376/2010 (European Commission, 2010) (yellow fat spreads, dairy products, mayonnaise and salad dressings), and that the claimed effect can

also be achieved with other matrices (rye bread, oatmeal, fermented soy milk based products, drinkable and spoonable yoghurt-type products, and oat based milk drinks). In response to an EFSA request to provide scientific information and/or justification to substantiate that a 12 % reduction of LDL-cholesterol is achieved with 3 g/day plant stanols as plant stanol esters added to other food matrices, the applicant analysed the 57 plant stanol ester studies identified by Musa-Veloso et al. (2011) and provided results for the mean and range of the relative LDL-cholesterol lowering effect per gram of plant stanol esters for 31 margarine treatment arms (4.60 %; 1.92 - 9.31) and for 11 treatment arms which used other food matrices (mayonnaise, dairy products, salad dressing) specified in Regulation (EC) No 376/2010 (4.12 %; 1.32 - 9.68). The Panel notes that all 18 treatment arms analysed in the meta-analysis used matrices specified in Regulation (EC) No 376/2010, and that no human study has been provided by the applicant to demonstrate that 3 g/d plant stanols consumed with other food matrices can lower blood LDL-cholesterol by 12 %.

The Panel reiterates its previous conclusion (EFSA, 2009) that while plant sterols/stanols added to foods such as margarine-type spreads, mayonnaise, salad dressings, and dairy products such as milk, yoghurts, including low-fat yoghurts, and cheese have been shown consistently to lower blood LDL-cholesterol levels, the size of the cholesterol-lowering effect of plant sterols/stanols added to other food formats is less well established.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- plant stanol esters at a daily intake of 3 g plant stanols (range 2.7 g to 3.3 g) in matrices approved by Regulation (EC) No 376/2010 (yellow fat spreads, dairy products, mayonnaise and salad dressings) lowers LDL-cholesterol by 11.4 % (95% CI: 9.8 – 13.0).
- the minimum duration required to achieve the maximum effect of plant stanol esters on LDL-cholesterol lowering is two to three weeks.
- while plant stanol esters added to foods such as margarine-type spreads, mayonnaise, salad dressings, and dairy products such as milk, yoghurts including low-fat yoghurts, and cheese have been shown consistently to lower blood LDL-cholesterol levels, the size of the cholesterol-lowering effect of plant sterols/stanols added to other food formats is less well established.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on plant stanol esters and blood cholesterol pursuant to Article 14 of Regulation (EC) No 1924/2006 (Claim serial No: 0307_FI). June 2011. Submitted by Raisio Nutrition Ltd.

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GLOSSARY / ABBREVIATIONS

CHD	Coronary heart disease
LDL	Low density lipoproteins